detecting complexes formed by specific binding between the antibody or fragment and VLA-4 present in the target sample.

29. (Amended) A method of treating [an inflammatory disease] multiple sclerosis in a patient comprising administering to the patient a therapeutically effective amount of the pharmaceutical composition of claim 25.

Please add the following new claim:

31. The method of claim 29, wherein the patient is already suffering from multiple sclerosis and the administration of the pharmaceutical composition at least partially arrests the symptoms of the disease.

REMARKS

Claims 1-26, 29 and 31 are pending. Claims 27-28 and 30 have been cancelled without prejudice. The paragraph numbering of the office action is used in responding to the Examiner's comments.

15. Applicants will supply formal drawings on notification of allowable subject matter.

17. The Examiner says that it is not clear whether claim 24 is directed to a computer, a mathematical algorithm, a computer program or a physical representation. In response, the Examiner is advised that claim 24 is directed to a computer (*i.e.*, hardware) programmed in such a manner to display the recited three dimensional image. Although Applicants believe the claim was unambiguous as written, the format of the claim has been changed such that the word "computer" is now used in the body of the claim. Thus, the claim is directed to statutory subject matter and the rejection should be withdrawn.

18. The Examiner has rejected claims 25-30 for lack of sufficient evidence utility of *in vivo* therapeutic and diagnostic utility. The Examiner takes the view that therapeutic methods

are unpredictable in the absence of *in vivo* clinical data because of inactivation of the active ingredient, targeting difficulties or side effects. The Examiner states that it is not clear that *in vitro* results accurately reflect the relative superiority of the claimed therapeutic strategy. The Examiner cites Harris as disclosing difficulties in the use of rodent antibodies, and Emery as disclosing that problems of immunogenicity may occur even with humanized antibodies. The Examiner further states that effects of antibody specificity, binding constants, tissue penetration, clearance rates and mode of action of effector are not necessarily predictable. The Examiner cites Harlan as disclosing that adhesion-based therapies are a long way to product. The Examiner also states that it is not clear whether the claimed antibodies can be used to treat ongoing disease or whether they are effective only in terms of prevention. The Examiner cites Dijkstra as disclosing that no immunosuppressive treatment has yet justified widespread administration to MS patients.

Claim 26 has been amended to refer to an *in vitro* method of diagnosis, so the rejection is moot with respect to this claim. With respect to the pharmaceutical and method of treatment claims, an extract from commonly owned PCT application filed January 25, 1995, Attorney Docket No. 15270-001410PC (a CIP of the present application), is attached providing *in vivo* data that the claimed humanized antibodies are effective in both prophylactic and therapeutic treatment of an animal model system simulating multiple sclerosis and the claims have been amended to focus on methods of treating this disease.

It is respectfully submitted that many of the issues raised in the office action result from the application of an unduly high standard of utility. As noted in the new draft guidelines on utility, the case law establishes that data generated from animal models are almost invariably sufficient to establish utility. Further, the law does not require Applicants to supply proof beyond a reasonable doubt; rather the burden is on the Examiner to establish by a preponderance of the evidence that the

utility asserted would be considered incredible by persons skilled in the art. *In re Irons*, 144 USPQ 351, 354 (CCPA 1965).

The Examiner's remarks on possible inactivation, targeting difficulties or side-effects might have had some validity if the claimed utility were supported only by *in vitro* assays. However, the attached extract from the PCT application (Attorney Docket No. 15270-001410PC) provides data indicating that none of these problems prevented efficacy in a protypical laboratory animal (*i.e.*, the guinea pig) suffering from EAE, a syndrome which has long been recognized to simulate many of the symptoms of MS in humans. (*See* Paterson in *Textbook of Immunopathology* (eds. Miescher et al., Grune & Stratton, NY, 1976), pp. 179-213). The Examiner has advanced no specific reasons that factors such as inactivation and targeting would pose insuperable difficulties in humans when they do not in a model animal. In the absence of specific reasons, a skilled practitioner would regard the demonstrated efficacy in a laboratory animal as reasonably predictive of that in humans, which is all that is required for patentable utility.

Specifically with respect to the alleged immunogenicity of the claimed antibodies, it is noted that even in clinical trials of mouse antibodies, the induction of a HAMA response has not usually been so immediate or universal to preclude obtaining any benefit from antibody administration. For example, the murine OKT3 antibody has proved beneficial to kidney transplant patients notwithstanding occasional reverse reactions (*see* Harris, *TibTech* 11:40-42 (1993)) and has been approved by the FDA for this purpose. Moreover, clinical trials of humanized antibodies have indicated that immunogenicity is very much reduced or even eliminated compared with mouse antibodies. *See* Anasetti et al., *Blood* 84:1320-1327 (1994); Caron et al., *Blood* 83:1760-1768 (1994). As even the cited Emory reference notes "the reduction in immunogenicity [of humanized antibodies] has been encouraging." (at p. 245).

The quoted sentence from Harlan should be attributed very little weight in assessing the utility of the present methods.

This quote was taken from an article published in April 1992, at a time when "moving into animals" had been held back because of lack of reagents. Harlan's other remarks indicate that his reservations largely stem from the unreliability of *in vitro* experiments and absence of animal models at the date of the article. It is impossible to tell from this article how Harlan would have assessed the likelihood of clinical utility from the present data showing efficacy in a mammal.

With respect to the Examiner's citation of Dijkstra for the proposition that no immunosuppressive treatment is presently in widespread use for treating multiple sclerosis, it is submitted that a proposed therapeutic regime need not be either of widespread application, or in present operation for patentable utility. It is sufficient that the claimed agent show evidence of pharmacological activity in a recognized screening system. *Nelson v. Bowler*, 206 USPQ 881 (CCPA 1980). Such is abundantly demonstrated by the data in the attachment.

For these reasons, it is respectfully submitted that the rejection should be withdrawn.

19. Rejections under §112, first paragraph.

A. The specification is objected to for alleged failure to teach how to use the *in vivo* methods of claims 25-30. The reasons advanced for the rejection are the same as those for the §101 rejection. Thus, Applicants respond as above.

B. The Examiner says that a deposit of the mouse 21.6 antibody is required. Applicants respectfully disagree. The application presents complete amino acid sequence data for the variable domains of the mouse 21.6 antibody and for exemplary humanized versions thereof. From this data, one could readily synthesize nucleic acid constructs encoding either the mouse 21.6 antibody or humanized versions thereof (as did the present inventors).

20. The claims stand rejected under §112, first paragraph, for the same reasons as the specification. Applicants respond as above.

21. Rejections under §112, second paragraph

A. Claim 1 has been amended to recite "second group" as suggested by the Examiner.

B. Claim 1 has been amended to refer to "humanized immunoglobulin" as suggested by the Examiner.

C. The Examiner says that use of "21.6 immunoglobulin" alone renders the claims indefinite. In response, the claims have been amended to recite the SEQ. ID. Nos. for the light and heavy chain variable domains of the 21.6 immunoglobulin.

D. The recital of "VLA-4 ligand" in claim 1 has been amended to --VLA-4-- as suggested by the Examiner.

E. Claims 17, 22, 23 and 26 have been amended to recite --antigen-binding fragment-- as suggested by the Examiner.

F. The Examiner suggests that claim 26 be broken down into *in vitro* and *in vivo* diagnosis for improved clarity. Claim 26 has been amended as discussed in the §101 rejection to refer to *in vitro* diagnosis.

24. Claim 24 is rejected under §102b as being anticipated by a Silicon Graphics Iris 4D workstation running under the UNIX operating system and using the molecular modelling package Quanta™, as disclosed by Kettleborough. This rejection is respectfully traversed. The presently claimed computer is distinguished from Kettleborough not only in the manner of use, but also because the different manner of use requires that the computer be programmed with different information. For example, to display a three-dimensional representation of the humanized antibodies specified by claim 1, the computer must at the very least have received sequence data for the mouse 21.6 antibody, and additional data pertaining to the modelling of the antibody and selection of amino acids for substitution. These data are stored as magnetic patterns in the computer. The presence of

these magnetic patterns confers a physical distinction between the presently claimed computer and that discussed by Kettleborough rendering the rejection under §102b improper.

25. Claims 1-27 and 29 stand rejected under §103 as obvious over Monshizadegan and Wayner WO 91/03252 in view of Queen and Kettleborough. The Examiner states that Monshizadegan teaches the 21.6 antibody and its ability to inhibit $\alpha 4$ integrin. The Examiner states that Wayner discusses other antibodies to the $\alpha 4\beta 1$ antigen, and that it is not clear whether there are critical differences between the 21.6 antibodies and Wayner's antibodies. The Examiner says that Queen and Kettleborough teach humanizing an antibody including vectors, nucleic acids, modelling processes and computers. The Examiner takes the position that one would have been motivated to select and evaluate the efficacy of humanizing VLA-4 specific antibodies including the 21-6 monoclonal antibody as a diagnostic and therapeutic tool in treating human diseases, and would have had a reasonable expectation of success. This rejection is respectfully traversed.

1. The Invention

The invention as specified by claim 1 is directed to humanized antibodies to VLA-4 derived from the mouse 21.6 antibody. Also claimed are methods of treating multiple sclerosis using the claimed antibodies. New dependent claim 31 specifies that the patient is already suffering from multiple sclerosis and the treatment at least partially reverses the symptoms.

The attached extract from the PCT application (Attorney Docket No. 15270-001410PC) provides evidence that the claimed humanized antibodies are effective in delaying and reversing clinical symptoms in an animal model simulating multiple sclerosis in humans. Specifically, treatment with humanized antibody of guinea pigs having established disease resulted in significant reversal of clinical symptoms, significant gain of

weight, significant reduction of leukocyte infiltration into the CNS and significantly increased platelet count. No leukopenic side effects were observed. Treatment with an equal dosage of mouse 21.6 antibody also resulted in significant delay of clinical symptoms but did not result in a statistically significant reversal of symptoms.

The extract from the PCT application also indicates that the 21.6 antibody has other useful properties rendering it particular suitable for therapeutic methods. The extract indicates that the 21.6 antibodies block binding of VLA-4 to VCAM-1 irrespective of the presence of Mn^{2+} (an activator of VLA-4) and irrespective of the concentration of VCAM-1. By contrast a commercially available antibody to VLA-1 (L25) showed little blocking at high [Mn^{2+}] or [VCAM-1]. The extract notes that both activation of VLA-4 and upregulation of VCAM-1 are likely to occur in an *in vivo* inflammatory response. Thus, the properties of the 21.6 antibody in blocking irrespective of [Mn2+] and [VCAM-1] render it particularly suitable for *in vivo* therapeutic administration.

2. The Cited Art

Monshizadegan discusses the mouse 21.6 antibody and reports that the antibody is able to inhibit binding of U937 or BAL cells bearing VLA-4 to VCAM-1. The antibody also inhibited binding of U937 cells to fibronectin but did not inhibit binding of BAL cells to fibronectin.

Wayner reports that leukocytes can attach to endothelial cells via the $\alpha 4\beta 1$ receptor. Wayner also discusses four mouse antibodies to $\alpha 4\beta 1$, which are reported to inhibit binding of leukocytes to a 38 kDa fragment of fibronectin.

Kettleborough discusses the humanization of a mouse monoclonal antibody to EGFR. Kettleborough produced a total of nine heavy chains having different variable region framework amino acid substitutions, and two light chains having different variable region framework amino acid substitutions. The different chains differed widely in binding affinity, with one substitution (position H71) having a large effect and other

substitutions having moderate or small effects. The best combination of heavy and light chains had a lower avidity than the starting mouse antibody. The authors describe the large effect of the H71 substitution as "surprising," and the roles of substitutions at positions 48, 66 and 67, as "difficult to explain" (p. 782, first column, 4th and 6th paragraphs).

Queen discusses generalized criteria for humanizing an antibody and a humanized anti-tac antibody made by the method.

3. The Cited Art Distinguished

A. No Motivation to Select 21.6 Antibody

The claimed humanized antibodies possess several unexpected and desirable properties for *in vivo* administration (capacity to reverse established disease and [Mn^{2+}]- and [VCAM-1]- independent blocking). The cited art would have provided no suggestion to select the mouse 21.6 antibody as a starting material with which to commence the laborious and expensive process of producing humanized anti-VLA-4 antibodies having these desirable properties.

The potential repertoire of mouse anti-VLA-4 antibodies from which to select a starting material would have been indefinitely large. Wayner discusses four mouse monoclonal antibodies other than 21.6 and Yednock (of record) describes an additional three, and Elices, *Cell* 60, 577-584 (1990) describes a further two antibodies. As noted in the attached extract, an additional antibody, L25, is commercially available from Becton Dickinson. Further, any number of other mouse antibodies could have been produced by conventional methods.

This large repertoire of antibodies would have been expected to differ substantially in functional properties. Even when antibodies are elicited in one strain of mice against a small haptenic antigen such as azophenylarsonate (MW 217), the resulting antibodies have different sequences and different binding characteristics. *See* Capra et al., *Immunology Today* 3, 332-339 (1982). If antibodies are elicited against a much larger and structurally more complex heterodimeric protein, the

diversity of properties is expected to be much greater. This expectation is confirmed by the observation that the α4 subunit of VLA-4 has at least two distinct ligand-binding domains (VCAM-1 and fibronectin) and that different antibodies have different capacities to block these respective bindings (*see, e.g.*, Yednock et al. at p. 65, column 1, 3rd paragraph). For example, the antibodies reported by Wayner block fibronectin binding, those reported by Yednock block fibronectin binding or both fibronectin and VCAM-1 binding, and one of the antibodies discussed by Elices (HP1/3) was reported to block only VCAM-1 binding. The functional complexity of the VLA-4 antigen is also evidenced by the wide spectrum of events mediated through it, including T-cell proliferation, cytokine production, promotion or inhibition of cell death, regulation of a T-cell gelatinase gene, regulation of monocyte inflammatory mediators, triggering of tyrosine phosphorylation, as well as stimulation of transendothelial migration of monocytes and lymphocytes. Lobb & Hemmler, α4 *Integrins in Vivo*, at p. 1723.

The cited art would have provided no indication which, if any, mouse antibody to VLA-4 could have been humanized to possess similar functional properties to the claimed antibodies in reversing symptoms of disease *in vivo*. As the Examiner has noted at p. 3, 3rd paragraph of the office action, the capacity to reverse the symptoms of disease is not expected simply because an antibody can delay disease when administered prophylactically. Even if one were to have screened a large number of mouse antibodies for the property of reversing disease *in vivo*, it was not known whether a suitable candidate would have been identified.

The cited art would also have failed to indicate which, if any, mouse antibody to VLA-4 would possess the property of [Mn^{2+}]- and/or [VCAM-1]-independent blocking capacity. The cited art did not suggest that antibodies should be screened for these properties or indicate how many antibodies would have to be screened to identify a candidate having the requisite properties.

Because the cited art provided no motivation to select the 21.6 antibody for humanization, and it was unknown, which, if any, other antibodies could have been humanized to have the same desirable properties, it is respectfully submitted that the rejection should be withdrawn.

B. Unexpected Efficacy Against Established Disease

As previously noted, the claimed humanized antibodies cause a statistically significant reversal of disease symptoms in a an animal model simulating multiple sclerosis in humans. Such a result has not previously been reported for any antibody to VLA-4. As the Examiner agrees in the §101 rejection, this result could not have been predicted merely from the ability of antibody to act prophylactically in preventing onset of disease. The acquisition of hitherto unknown useful functional properties in the claimed humanized antibody must be considered an unexpected result indicative of patentability.

C. Claims 14, 15 and 16: Unexpected Binding Affinity

Each of these claims is respectively directed to a humanized antibody comprising light and heavy chain variable regions of designated sequence (Ha/La, Hb/La and Hc/La, respectively). The Ha, Hb and Hc chains have 5, 6 and 6 variable regions framework substitutions and the La chains has seven such substitutions. Each of these antibodies binds to VLA-4 at least as well as antibody having the unmodified murine variable regions (*see* Example 7, §4). Further, the Ha/La version has been show to be capable of reversing the symptoms of MS disease in an animal model at least as well as, and possibly better than the mouse antibody from which it was derived (*see, e.g.*, Figs. 15 and 16 of the attachment).

No reasonable expectation would have arisen that the antibodies having precisely the variable region sequences now claimed would exhibit the same or better binding affinity than the mouse monoclonal antibody from which their CDR regions were obtained. Although generalized criteria are helpful for

identifying candidate variable regions substitutions, the precise combined effects of simultaneously substituting 12 or more amino acids are not predictable in advance. Indeed, even with the benefit of hindsight provided by measuring the binding affinity of modified antibody chains, the precise effects of substitutions are described by Kettleborough as "surprising" or "difficult to explain" (p. 782, first column, 4th and 6th paragraphs).

The transition from a murine to a humanized antibody involves a compromise of competing considerations. To minimize immunogenicity, the immunoglobulin should retain as much of the human acceptor sequence as possible. However, to retain authentic binding properties, the immunoglobulin framework should contain sufficient substitutions of the human acceptor sequence to ensure a three-dimensional conformation of CDR regions close to that in the mouse donor immunoglobulin. Consistent with this compromise, many humanized antibodies produced to-date have showed lower binding affinity than their murine counterparts. *See, e.g.*, Jones et al., *Nature* 321: 522-525 (1986); Shearman et al. (1991), *J. Immunol.* 147: 4366-4373; Kettleborough (1991), *Protein Engineering* 4: 773-783; Gorman et al. (1991), *Proc. Natl. Acad. Sci. USA 88*:4181-4185; Tempest et al. (1991), *Biotechnology 9*:266-271. In summing up the state of the art, Chiswell & McCafferty state:

> [D]espite our knowledge of the structure of the antibody molecule...humanized antibodies do not usually have the affinity of the original rodent mAb. In practice, success is usually claimed for an antibody with only 33-50% of the original affinity.

TibTech 10, 80 (March 1992).

In this state of knowledge in the art, the production of humanized antibodies having the same or better binding properties to the mouse antibody from which they were derived clearly represents an unexpected result.

Claim 16

The Hc chain of the antibody specified by claim 16 contains a tyr-phe amino acid substitution of the CDR3 region to make the CDR loop more closely resemble the VCAM-1 ligand (*see* specification at p. 35, 4th paragraph). This substitution is contrary to the usual practice in making humanized antibodies, in which substitutions of CDR regions are avoided because of the likelihood that alterations to these critical regions will substantially reduce binding affinity. As Kettleborough notes, even "relatively conservative changes in the FR residues can strongly influence antigen binding" (at p. 782, column 1). Changes in the CDR regions are expected to have even greater influence on binding affinity because the CDR residues generally directly contact the antigen. The cited references do not suggest making a substitution within a CDR region to confer a conformation more closely resembling a ligand. Moreover, it could not have been expected that the unmodified CDR3 of the mouse 21.6 antibody would have shown a sequence similarity with the binding domain of VCAM-1, such that a substitution to increase the similarity might be advantageous. Because there was no motivation to make the claimed amino acid substitution in the CDR3 region of the mouse 21.6 antibody, the patentability of claim 16 is established on a still further ground.

26. Claims 28 and 30 stand rejected under §103 over the same references as claim 1-27 and 29, in further view of Yednock. The Examiner says that Yednock teaches the use of VLA-4 specific antibodies in the treatment of experimental encephalomyelitis as a model of multiple sclerosis. The Examiner takes the view that one would have been motivated to select and evaluate the efficacy of humanized VLA-4 specific antibodies including the 21.6 antibody in the treatment of inflammatory diseases with a reasonable expectation of success.

In response, it is noted that the claimed methods are nonobvious for at least the same reasons as those discussed above. In addition, the efficacy of the method of claim 31

(treating established MS disease) could not have been predicted with a reasonable expectation of success from the Yednock reference. This reference discusses only the protective effect of an antibody administered prior to the onset of EAE syndrome and provides no data showing that an antibody to VLA-4, humanized or otherwise, is effective to reverse symptoms of established disease. As the Examiner agrees in his §101 rejection, the capacity to reverse the symptoms of disease is not expected simply because an antibody can delay disease when administered prophylactically. The attached data reveal for the first time that concentration of invading leukocytes can be reduced in the CNS after onset of disease, and that this reversal is associated with improvements of clinical symptoms, weight gain, and increased platelet counts. In view of this unexpected result, as well as the reasons discussed for the other claims, the rejection should be withdrawn.

Respectfully submitted,

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Attachment:
Extract from PCT application

15270\14.R01